GROWTH OF PLECOPTERA (STONEFLY) NYMPHS AT CONSTANT, ABNORMALLY HIGH TEMPERATURES[1]

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Abstract.— Six species of Plecoptera were maintained at four different temperatures, which were constant and higher than occurred in the natural habitat, and three species at two different day lengths. Each animal was weighed each day or each week. Weight of two species in the wild was monitored from periodic collection.

The weight of each animal fluctuated rhythmically, changing about five percent every five days. These short-term fluctuations probably resulted from changes in water content. Molting occurred when a peak weight was predicted from the cycle and involved temporary gain of about 20 percent in weight. Growth probably stopped for some time before molt and was most rapid just afterward. Many animals died at molt.

The time before death was less for univoltine species than for those with longer life cycles. Plecoptera collected in winter from water near 0 C lived for shorter times than did those collected in autumn from water near 10 C. Two species died sooner at higher temperatures and one died sooner with shorter day lengths.

Growth in the laboratory was generally slower than in nature. One species grew faster, while three grew more slowly at higher temperatures. One species grew faster under long- than short-day conditions.

Premature emergence, expected at the higher temperatures, did not occur, except in one animal.

Calefaction, or abnormal warming, can alter the life span of some aquatic insects. Some die soon after being experimentally exposed to higher than normal temperatures. Others acclimatize and may emerge prematurely (Nebeker and Lemke, 1968; Nebeker, 1971). Effects of constant, higher - than - normal temperatures on the growth and development of aquatic insects are, however, little understood.

Temperature changes could be important cues to growth and seasonal emergence. Species living in or near springs, where the temperature is relatively constant and does not become as cold as in neighboring streams, frequently emerge unseasonably (Thorup, 1963). Alternately, temperature changes are necessary for normal development of some insects (Hodson and Rawy, 1956). Such changes lead to considerable biochemical restructuring on a seasonal basis (Somero and Hochachka, 1971) and can also affect gene expression and the phenotype of insects (Sanderson, 1910; Wigglesworth, 1965; Waddington, 1957). The elimination of temperature changes could, therefore, interfere with normal development.

Abnormally high temperatures could have long-term cumulative effects on growth and development (Sander, 1910; Richards, 1956, 1957). Growth could be faster than normal because chemical rates generally accelerate, or slower because metabolic equilibria are upset (Ludwig, 1910). The effect on growth could in turn influence development, emergence, and adult function.

Understanding the effects of temperature on growth and development of aquatic insects would be useful in appraising the effects of thermal pollution, because larval insects are an important component of aquatic environments, particularly streams (Hynes, 1970). The purpose of this research was to elucidate the growth patterns of some species of Plecoptera maintained in the laboratory at different temperatures. Stonefly nymphs were chosen because they develop in winter and spring when the stream temperatures are often near freezing and because they are abundant. Three carnivorous and three herbivorous species were collected in autumn 1972 and established in the laboratory at four different temperatures and two different day lengths. Some were examined and weighed daily; others, weekly. Growth patterns of individuals were plotted, and statistical comparisons were made between groups of animals under different conditions.

Materials and Methods

Some features of the species studied are outlined in Table 1. All were collected in October, when stream temperatures

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Table 1. Collection sites and activity cycles of species studied.

	Family	Time of Normal Emergence†	Years in Life Span	Collection Sites
Nemoura cinctipes	Nemouridae	Jan.-May	1	Mill Creek, Salt Lake Co.
Pteronarcella badia	Pteronarcidae	May-Sept.	1	Upper Provo R., Wasatch Co.
Pteronarcys californica	Pteronarcidae	Apr.-Aug.	>1	Lower Provo R., Utah Co.
Arcynopteryx signata	Perlodidae	June-July	1	Mill Creek, Salt Lake Co.
Acroneuria pacifica	Perlidae	Mar.-Sept.	>1	S. Fork Provo R., Utah Co.
Claassenia sabulosa	Perlidae	June-Aug.	?	Weber R., Summit Co.

†Gaufin, Nebeker, and Sessions, 1966

were 10-12 C, and the experimental groups were established in the first part of November, except where noted below.

The specimens were maintained separately in perforated plastic (polyethylene) drinking cups 9.5 cm in depth and 7 cm in top diameter, tapering to 5 cm at the bottom. The cups were suspended and were about half submerged in stainless steel aquaria approximately 100 cm long, 17 cm wide, and 15 cm deep and having a 25.5 L^3 volume. Nine such aquaria were suspended in two refrigerated water baths; four in a bath at 9.5 C and five in another bath at 13 C. Each aquarium was heated with a thermostatically regulated element. Tap water flowed through each aquarium at about 0.4 L/min. (or about one aquarium volume/hour). Each aquarium was aerated with filtered compressed air and agitated with a paddle wheel that mixed the water in the aquarium and caused a predominantly up-and-down oscillation of water in the cups. Concentration of dissolved oxygen remained greater than 90 percent of saturation in the cups. Temperature was maintained with a standard error of $\pm$ 0.02 C and a range of about $\pm$ 0.5 C, at 10, 12, 14, or 16 C. These aquaria were lighted from about one meter above with two fluorescent bulbs on a 12-hr. light, 12-hr. dark cycle. Ambient room light, which was not excluded, varied somewhat.

In a separate experiment to estimate the effect of light periodicity, three species were maintained in cups suspended in plastic aquaria flushed with aerated running water (1 vol./hr.) but with no paddle wheel (dissolved O_2, > 90 percent saturation). These were in a walk-in cold room that maintained the water temperature at 11.5 C within the same limits as in the other aquaria. They were illuminated with fluorescent lights (2 bulbs) that delivered about 400 Lux at the water surface on either a 12L, 12D (long-day) or a 6L, 18D (short-day) periodicity (Beck, 1968).

One or several small stones and several decaying leaves (cottonwood, *Populus angustifolia;* or maple, *Acer grandidentatum*) were kept in each cup. Herbivorous species were also supplied a few pellets of Purina® rabbit chow every few days, and the carnivorous ones were kept supplied with a mixture of small aquatic organisms, including amphipods, chironomids, ephemeroptera, oligochaetes, and flatworms collected from near a local fish hatchery.

Changes in weight were monitored either each day between 1 and 3 PM or each week on Thursday between 1 and 5 PM. Each animal was gently blotted with a Kimwipe® (Kimberly-Clark Corporation), air-dried for half a minute, and weighed to the nearest 0.1 mg on a Mettler H6T balance. The standard error of the method, determined by repeatedly weighing the same animals about 20 times in an hour, was about 0.002 of the mean for animals about 200 mg, about 0.005 of the mean for ones about 150 mg, and about 0.01 of the mean for animals about 30 mg. Repeated weighing revealed a gradual but statistically significant decrease in weight of each animal even though they were returned to the water between each determination, so the standard error of single weighings each day or week may have been less than for about 20 weighings an hour.

Natural growth rates were calculated for *Pteronarcella badia* and *Arcynopteryx signata* from approximately monthly collections of 22 specimens of each species from the initial site. The animals were weighed as above, within several hours of collection, and growth was determined as the rate of change of the average weight.

The data were evaluated by regression

analysis (Rao, 1958; Bailey, 1959; Alder and Roessler, 1968), using a Hewlett Packard 9100 B computer and programmed procedures supplied by the manufacturer. Growth trends of each individual were analyzed from weight data by determining the correlation coefficient (r) and, from it, the possibility that changes in weight were correlated linearly with time, according to the calculated slope (m) which intercepted the axis representing weight at the hypothetical initial weight (b) independent of fluctuations at the beginning of the observations. Growth was judged to be positive, negative, or insignificant from the correlation coefficient ($P > .05$), and its magnitude was determined from the slope. Absolute growth values, in weight units, were converted to relative ones for comparisons between animals by determining the rate of change as a percentage of the averaged initial weight ($m \times 100/b$). The effects of various conditions were then evaluated by t-test or regression analysis, using the relative growth rates.

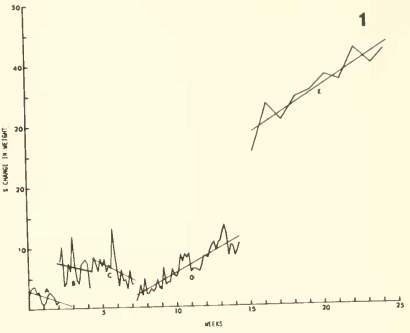

Fig. 1. Weight changes of a *Pteronarcys ca fornica* kept under various conditions. This ind vidual was collected in December and acclimati d to laboratory conditions for 15 days without foo It was then weighed daily while being starved A 14 days), fed rabbit chow B (15 days), and the leaves and rabbit chow C (21 days), while being ept at 10 C. It was then changed to 16 C an weighed daily while being fed leaves and rabbit how D (50 days) and finally weighed once eac week for 10 weeks (E). It did not molt and w s still alive after the 170-day observation period.

Short-term Fluctuations in Weight

The weight of all stoneflies examined every day increased and decreased rhythmically, typically varying 3 to 25 percent of the body weight about every 4-6 days (Table 2; Figs. 1, 2, and 3). The magnitude of weight difference between high and low periods exceeded the probable error due to the method, and the trends apparent in the plotted data indicate that the fluctuations were not merely artifacts of the method. There was no apparent correlation between the rhythmic pattern and environmental conditions, nor were the cycles of different animals in phase with each other, even though they were in the same aquarium.

It was hypothesized that periodic feeding behavior caused the observed rhythmic weight changes. To test this, ten *Pteronarcys californica* (collected 5 De-

Table 2. Periodic variations seen with daily weighings (Average ± SE) calculated from the first six cycles.

	No. of animals	Period (days between peaks)	Amplitude*
P. californica	5	5.9 ± .4	3.3 ± .3
P. badia	4	4.5 ±1.0**	5.4 ±1.6
A. pacifica	5	5.0 ± .4	7.2 ±1.0***
C. sabulosa	1	4.7	5.7
A. signata	1	6.3	12.3

*Difference between peaks and troughs, expressed as percentage of average weight.
**Significantly ($p < .05$) different only from *P. californica*
***Significantly ($p < .001$) different only from *P. californica*

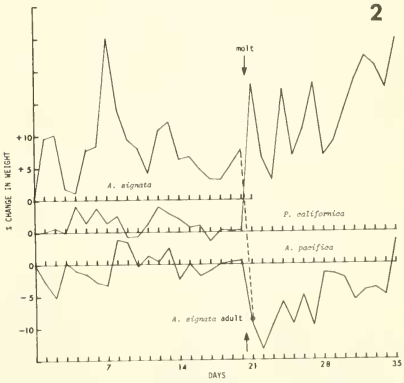

Fig. 2. Daily weight changes before and a er molting (*Pteronarcys californica* and *Acroneuri pacifica*) and before emergence (*Arcynopteryx si nata*). The molted cuticle was found and the an mal weighed about six hours after the molt occu ed.

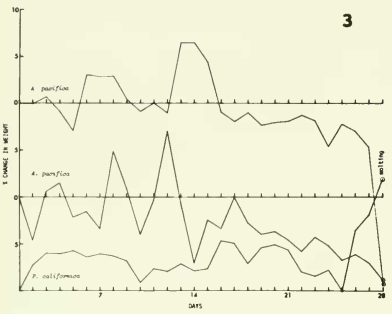

Fig. 3. Daily weight changes before death.

cember 1972) were starved for 15 days without weighing; then weighed every day for 14 days, while starving; then fed either Purina rabbit chow or decaying leaves and weighed daily for 15 days; and, finally, fed the other food and weighed daily. Starvation did not eliminate the rhythmic weight changes (Fig. 1), although all the animals were losing weight. The periodicity remained unchanged upon feeding either or both foods. The amplitude was not affected significantly ($P > .05$) when leaves were supplied but was significantly increased over the starvation level after feeding rabbit chow, either alone or with leaves (Table 3).

The rhythmic weight changes could have been associated with molting. Weight changes during molting were observed in a few specimens. Of the 16 animals weighed daily, five molted one time successfully, one emerged, and six died molting. One *Ps. californica* and one *Acroneuria pacifica* were weighed during a successful molt. The *Ps. californica* was 24.4 percent heavier at the time of molting than just before, and the *A. pacifica* was 19.0 percent heavier. The ones that died molting were all considerably heavier than they had been (Table 8). Molting generally occurred when the pattern of weight change indicated that a peak weight was due to occur. After molting, the period between weight maxima was usually shorter and the amplitude greater than it had been before the molt; but after several cycles, the pattern began to resemble the premolt condition again.

The one animal on a daily weighing schedule that emerged (an *Ar. signata*, Fig. 2) also molted and emerged at a peak in the weight cycle. The adult was 25 percent lighter than the nymph. Its gut was empty.

The frequency of weighing affected the weight of stoneflies. Animals weighed a number of times in an hour lost weight during the course of the observations. Five animals that had been weighed every day gained weight appreciably when the weighing frequency was reduced to once a week. The growth rate (m) was usually slightly greater with the less frequent weighings, but the change in rate was not statistically significant (Fig. 1). Handling could have caused the animals to contract and expel water or gut contents, and it likely interfered with feeding patterns.

Long-term Growth Patterns

The stoneflies under observation lived for various periods. Those that lived a month or more either gained, lost, or remained the same weight during their

Table 3. Effects of food on periodic variation, seen with daily weighings of *P. californica* (average ± SE).

	N	Period (days between peaks)†	Amplitude (difference between peaks & troughs, expressed as a % of average wt.)	Gain after feeding (% of previous wt.)
Starved	10	5.1 ± 1.0	2.2 ± .3	
Fed leaves	3††	5.6 ± 1.0	3.1 ± .3	4.3 ± .3
Fed rabbit chow	4††	5.3 ± 1.2	5.3 ± .4**	11.8 ± .6
Fed both	7††	5.1 ± 1.2	4.4 ± .5*	8.7 ± .5‡

†Estimated from three peaks

††Three that did not respond to either food were excluded from these calculations

‡Only those fed rabbit chow after leaves responded

*Significantly different ($p > .01$) from starved group only

**Significantly different ($p > .001$) from starved group only

TABLE 4. Average (X ± SE (N)) Weeks of life.

	10 C	12 C	14 C	16 C	Long Day (11.5 C)	Short Day (11.5 C)
P. californica†	25.2±3.0(5)	17.2±2.1(6)	24.5±4.9(6)	23.8±4.6(6)		
P. badia	15.0±2.5(5)	17.7±1.3(6)	11.4±2.1(6)	10.7±1.6(6)	6.0±1.1(11)	6.7± .6(11)
N. cinctipes	4.7± .9(6)	6.2±1.2(6)	2.5± .3(6)	4.7± .9(6)		
A. pacifica	18.9±5.8(5)	16.5±3.5(4)	18.0±4.3(6)	14.2±4.3(5)	15.6±1.9(11)	11.1±2.6(11)
C. sabulosa	17.0± .1(2)	17.5±1.5(2)		9.7±1.1(3)		
A. signata	9.5±3.1(4)	9.3±1.7(4)	2.7± .6(7)	2.8± .4(12)	3.4± .9(7)	2.7± .6(7)

†Eight *Ps. californica* and three *Ac. pacifica* were still alive at the time of writing, 35 weeks after the observations began.

life. Some individuals lost weight for part of their life and gained in another part. It was important to consider the history, length of life, and ultimate fate as well as the overall growth patterns in assessing the effects of temperature on growth rate.

Some species lived longer in the laboratory than did others (Table 4). In general the smaller, univoltine species, *Nemoura cinctipes*, *Pa. badia*, and *Ar. signata*, did not live as long as the larger species, *Ac. pacifica*, *Claassenia sabulosa*, and *Ps. californica*, which probably live for several years as nymphs (Table 1). *Pteronarcella badia* and *Ar. signata* lived for significantly ($P > .05$) less time at higher temperatures. *Pteronarcella badia*, *Ar. signata*, and *Ac. pacifica* kept in the light-control chambers died significantly sooner than their counterparts in the temperature experiments. The former were caught in winter from streams near 0 C and were kept without stirring. Day length had no significant effect on the life span of *Ar. signata* and *Pa. badia*.

Weight changes prior to death followed three distinctive patterns, which, in some specimens, could have been related to cause of death (Fig. 3, Table 5). Often death occurred during a recognizable molt. Sometimes the growth curve turned sharply up, as if molting, but there was no external sign of molting. More often, death followed a diminuation in the amplitude of cyclical weight changes and was not marked by any sharp change in weight. Two *Ps. californica* that did not change in weight upon being supplied with either food showed this pattern, and it may have reflected starvation. The third pattern was marked by a sharp loss of weight at death. Adults weighed less after emerging (Fig. 2), and such sharp terminal weight loss could have indicated unsuccessful emergence. The circumstances of death of all animals that died are presented in Table 5.

Periodic molting is a characteristic of Arthropod growth. However, many of the animals observed here apparently did not molt, while many others died in the process (Table 5). No *N. cinctipes* and only one *Ar. signata* were observed to molt. The occurrence and frequency of molting for the other species are enumerated in Table 6. Some molts were probably overlooked, but the analysis of growth curves suggests that most were detected by the presence of the cast cuticle.

The times until first molt, between molts, and until death for ones that did not molt are compared in Table 6. Time before the first molt (considering ones that molted successfully or that died molting) was quite variable and was probably a function of the condition of the animals at the time of capture. The time between molts was also highly variable. The average period before the first molt was not significantly different from the time between molts for any species. The average length of life of individuals that did not molt was also the same as the average period before the first molt and the period between molts. The average period before molt differed between species: *Ps. californica* = *C. sabulosa* > *Ac. pacifica* > *Pa. badia* (t-test, differences considered significant if $p > .05$). There was no cor-

TABLE 5. Circumstances of death (percentage of N).

	N	M†	1†	2†	3†	E†
P. californica	31††	8	26	50	15	0
P. badia	52	21	20	53	3	2
N. cinctipes	22	0	0	100	0	0
A. pacifica	40††	26	11	52	10	0
C. sabulosa	8	57	0	28	14	0
A. signata	42	2	11	74	11	2

†1 Growth curve turned up at death, as prior to molting
2 Growth curve continued unchanged until death
3 Growth curve turned down at death, as prior to emergence
M Died molting
E Emerged
††Four additional *P. californica* and two *A. pacifica* were accidentally killed.

TABLE 6. Occurrence and frequency of molts

	Number observed† molting				Weeks (average ± SE (range))		
	0x	1x	2x	3x	Before first molt	Between molts	Of life of ones not molting
P. californica	28	3	3	0	16 ± 3 (12-30)	15 ± 3 (10-19)	17 ± 2 (3-32)
P. badia	30	16	4	2	5 ± 1 (1-18)	7 ± 1 (1-11)	5 ± 1 (1-19)
A. pacifica	15	17	10	0	9 ± 1 (2-21)	9 ± 1 (2-18)	8 ± 1 (3-22)
C. sabulosa	1	7	0	0	15 ± 1 (13-17)		6 ± 3 (3- 8)

‡Molting recognized by finding a cast cuticle or found dead in the process of molting.

relation between size and the length of time before or between molts either within or between species. There was also no correlation between the length of time before molts and the temperature or light period for any species examined.

Changes in weight accompanied molting (Table 7). Most animals weighed during a molt (alive or dead) showed a dramatic increase over the premolt weight. Usually this increase was much greater than the longer-term increase (determined by comparing the average weight the month before molt with the average weight for the month afterward) and could have been associated with the mechanism of molting. Animals that molted several times, and thus apparently were adapted to laboratory conditions, grew appreciably between molts. Some of the ones that died without molting grew about the same amounts as did others between molts, suggesting that death could have resulted from failure to molt.

For each animal observed, the growth rate (calculated as the regression coefficient [m]), for the four weeks prior to molting usually was different from that for the month after molt (Table 8). There was a great deal of variability between individuals: some lost before and gained after; some gained before and lost after; some gained or lost more rapidly before than after; and vice versa. One *Ps. californica* lost 1.0 percent/week for 20 weeks, molted, and then gained 1.1 percent/week for 15 weeks. The growth patterns in Fig. 2 are from apparently normal animals that lived many months in the laboratory. The number that molted and lived at least a month afterward was small, so averages and limits (Table 8) do not indicate significant ($p > .05$) differences in average pre- and postmolt rates. There was no significant correlation with the controlled parameters of temperature or light.

The overall growth of each animal until death was evaluated by calculating the correlation coefficient (r) and the regres-

TABLE 7. Change in weights with molting.

	Percent change in weight (average ± SE (range)).									
	N	During molt†	N	With molt††	N	Between molts‡	N	Total, ones not molting‡‡
P. californica	4	26± 2(22-30)	6	25±5(9-41)	3	22.6±4.9(13.1-29.1)	28	19.4±10.1(-22.8-174.3)
P. badia	10	65± 6(36-85)	10	12±4(5-32)	5	9.8±5.7(-1.2-20.4)	30	21.6± 6.9(-25.1-144.4)
A. pacifica	12	22± 4(3-47)	12	2±2(-9-19)	8	15.6±5.5(0.7-48.6)	15	-3.8± 1.6(-14.4- 9.2)
C. sabulosa	4	36±14(18-78)	3	1±7(-8-15)	0		1	10.0

†Animals weighed during a successful molt or found dead in the process of molting. Weight during molt as a percentage of the last premolt weight.
††Average weight for the month after molt as a percentage of the average for the month preceding the molt.
‡Calculated from the growth curve. Slope multiplied by the time and expressed as a percentage of the intercept (i.e., the hypothetical initial weight).
‡‡Total growth calculated as m x weeks of life x 100/b.

TABLE 8. Growth rates (percentage change/week) before and after molt (m ± SE (range)).

		Month before molt	Month after molt
P. californica	6	-.16 ± .84 (-3.35 - 2.05)	2.79 ± .81 (.09 - 5.75)
P. badia	9	2.73 ± 1.34 (-2.37 - 10.69)	-.39 ± 1.29 (-9.76 - 5.75)
A. pacifica			
All	26	.54 ± .41 (-2.68 - 6.32)	1.17 ± .56 (-3.23 - 10.98)
Long day	7	.96 ± .99 (-3.68 - 3.46)	3.23 ± 1.20 (- .05 - 10.96)
Short day	5	-.02 ± .50 (-2.53 - 1.69)	-.30 ± 1.00 (-3.23 - 1.69)
C. sabulosa	3	.38 ± 2.07 (-2.71 - 4.33)	-2.04 ± .78 (-3.57 - -1.08)

sion coefficient (m) in 0.1 mg change/week. The overall growth rate was sometimes influenced strongly by the final phase of the growth curve as the animal expired. Growth during the first two months was therefore calculated separately. To simplify comparison between species and experimental groups (Table 4) the growth rate (m) was expressed as a percentage of the calculated initial weight (b, the intercept). The number of animals was so small and the range of values so great within each group that averages and standard errors are of little meaning. Regression analysis of rates of change against temperature, however, indicated that temperature had a significant effect on the growth rates of some animals (r significant at $p > .05$). The proportionate (percentage) effect of temperature on growth rate, exclusive of experimental error, is expressed as the coefficient of determination ($r^2 \times 100$) (Alder and Roessler, 1968). The relationship between difference in growth rate per degree Celsius is expressed by m.

These observations were apparently valid only for the animals collected in October and November, when the stream temperatures were still about 10 C. The few animals collected later in the year from colder streams died in a shorter time and generally grew at rates different enough from the others' that it seemed best to exclude them from the preceding calculations.

Light periodicity could also have affected development in the laboratory. Significantly more *Pa. badia* molted under long- than short-day conditions. There was no significant difference in length of life or growth rate between animals of either species held under long- or short-day conditions. *Acroneuria pacifica* lived longer under long-day conditions (15.5 ± 2.0 vs 10.2 ± 2.3 weeks) but molted sooner under short days (7.1 ± 1.5 vs 9.1 ± 1.5 weeks). Significantly more molted under long than short days. Their growth rate to death was significantly greater under long days (1.9 ± .8 percent/week) than under short days (-1.0 ± .3 percent/week) (Table 6). These animals were not strictly comparable to the ones used for the temperature experiments: the water in their tanks was not stirred, and they were collected later in the year (*Pa. badia* and *Ac. pacifica* in November and *Ar. signata* in February). *Pteronarcella badia* and *Ar. signata* kept under equal periods of light and dark died significantly sooner than ones under similar light and temperature conditions but collected earlier and kept in stirred water (Table 4).

Laboratory conditions were quite different from nature, so it was desirable to compare growth of laboratory and wild animals from natural populations where possible. *Pa. badia* and *Ar. signata* persisted at the initial collecting sites in populations of relatively uniform-sized individuals. Their growth in nature was quite linear for both species between October and May 9 (Fig. 4). The average percentage of weight increase per week of wild *Pa. badia* was 6.26 ± 0.5 (r = .998), and that of *Ar. signata* was 10.17 ± .07 (r = .938). Such an analysis was impractical with the larger species because of the simultaneous existence of several year classes and the effect of investigator selection. Wild *Ar. signata* emerged naturally in May (water temperature 5-8 C) and *Ps. californica*, *Pa. badia*, and *Ac. pacifica* in May (water temperature 10-11 C).

Discussion

Stonefly naiads varied in weight as time passed. Some of the variation was associa-

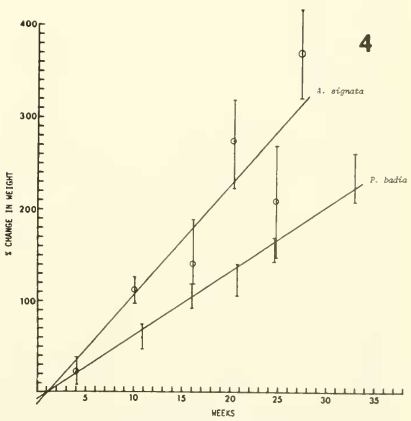

Fig. 4. Growth of *Pteronarcella badia* and *Arcynopteryx signata* in the stream. Means (with SE) were calculated from periodic samples of 22 animals and converted to percentage of the average initial weight.

ted with short-term fluctuation in state, while some represented long-term "growth." Variation in gut content rapidly altered the weight of experimental animals—as much as 10 percent or more (Fig. 1, Table 3). Cyclical weight changes, on the order of 5 percent variation every 5 days (Tables 2 and 3), occurred even in the absence of feeding and could have resulted from the amount of water in the animal, in either its gut or its tissue. This cyclical weight change could have been associated with molting. Each animal that was observed daily and that molted did so when a predicted peak weight should have occurred, and animals weighed considerably more when molting than just before or after the molt (Table 7). Such rapid weight gain could be responsible for splitting the old cuticle and filling out the new one (Wigglesworth, 1965).

The failure of molting was a common cause of death of animals in the laboratory. Many animals died during or just after molting. Others died at a time of peak weight, considerably above the previous average, as if about to molt (Fig. 3, Table 5). Most of the animals died without any molts except for *Ac. pacifica* and *C. sabulosa* (Table 6). On the average, those that did not molt died approximately when they should have molted, as judged from the average time before or between molts. Their average growth was also about the same as the average growth between molts of animals that survived in some species (Table 7). No *N. cinctipes* and only one *Ar. signata* were observed to molt, and their average life was less than for the other species (Table 4). It seems likely that failure of some aspect of molting was a common cause of death of stoneflies kept in the laboratory.

Relatively few animals lived long enough with repeated molts (Table 4) to be considered normal. The one *P. badia* that emerged (kept at 16 C) molted the first week in the laboratory (1 Nov.) with a 19 percent gain in weight, gained 28 percent more in the next 8 weeks, and emerged in January, 5 months prematurely. Its growth was almost linear from the beginning of the observations ($r = .998$) and slightly less ($m = 5.2$ percent/week) than the average for the species in nature. Most of the *P. badia* that molted successfully and then lived for at least a month gained weight prior to, and as a result of, the molt, but then lost weight (Tables 6, 7). Food may have been deficient, or the newly molted ones may have been less tolerant of laboratory conditions. Two *P. californica* that lived for the entire 35-week observation period molted twice each. Both lost weight the month before the first molt and showed no significant change the month before the second molt. Both gained considerable weight at both molts, remained at a higher weight, and continued to grow at an accelerated rate after the molt (Fig. 2, Tables 6, 7, and 8). They apparently did not feed before the molt, expanded in volume during the molt, and, at the larger volume, hardened and began to eat. The four *Ac. pacifica* that lived for the duration of the observations usually were not growing significantly prior to molt, expanded appreciably during the molt, but then declined to a weight considerably below the premolt weight within a day or so. They then grew rapidly for several weeks, until the previous weight was reached, after which they did not grow significantly again until after another molt. Some regressed at molting and lost weight overall (Table 7) (Fig. 2, Table 9). Probably their pattern was not normal but reflected subsistence in an unnatural environment (Beck and Bharadwaj, 1972).

The anticipated premature emergence (Nebeker, 1971) was not found. Of the animals collected in October, only one (*Pa. badia*) emerged and only a few died with a pattern of weight changes even suggesting emergence (Table 4). This could have been because the animals were collected early in the autumn and kept at constant temperatures and long-daylight conditions at or above those existing at the time of collection. Perhaps some environmental cue was absent. Or possibly the small plastic cups in which the animals were kept were too confining to permit normal behavior. A number of *P. californica* collected from streams near 0 C in January, acclimatized to 16 C, and kept communally in fish-breeding nets in the laboratory did emerge in March, three months before the wild population emerged naturally. One *Ar. signata*, collected in February, emerged 22 days after being put in a cup and kept at 10 C (Fig. 2).

The length of life of *Pa. badia* and *Ar.*

TABLE 9. Growth rates (m = percentage change in weight/week) and temperature, all data to death (upper half), and first two months only (lower half).

		10 C		12 C		14 C		16 C		Regression, growth rates against temperatures			
		N	$\bar{m}_{\uparrow} \pm$ SE	N	$\bar{m}_{\uparrow} \pm$ SE	N	$\bar{m}_{\uparrow} \pm$ SE	N	$\bar{m}_{\uparrow} \pm$ SE	N	m††	r	%r²
	all	5	.60 ± .23	6	.02 ± .48	6	1.00 ± .85	5	1.60 ± .86	22	0.25	.346	12
P. californica	+	3	.66 ± .25	2	1.23 ± .88	4	2.13 ± .65	5	1.60 ± .86				
	—	1	-.45	3	-.83 ± .25	1	-2.30	0					
	all	5	5.14 ± 1.53	6	2.79 ± .66	6	3.84 ± .68	7	1.53 ± 1.04	24	-0.49	-.417*	17
P. badia	+	4	3.58 ± .66	6	2.79 ± .66	6	3.84 ± .68	4	3.18 ± 1.07				
	—	0		0		0		1	-2.79				
	all	6	2.14 ± 2.47	4	3.11 ± 3.60	3	-5.54 ± 4.97	5	-.88 ± 1.45	18	-0.77	-.290	8
N. cinctipes	+	1	2.77	1	13.87	0		0					
	—	0		1	-1.32	1	-1.65	1	-2.21				
	all	4	2.63 ± 1.68	4	.50 ± 1.20	4	-.53 ± 1.34	12	-1.41 ± 1.72	24	-0.39	-.380	14
A. signata	+	0		1	3.93	0		1	1.63				
	—	0		0		0		0					
	all	5	.63 ± .48	4	.25 ± .45	6	.13 ± .42	5	.00 ± .59	20	-0.10	-.223	5
A. pacifica	+	3	1.65 ± .73	0		1	2.11	1	2.00				
	—	0		2	-.46 ± .12	0		1	-1.60				
	all	2	.51 ± .13	2	1.17 ± 2.15	0		3	1.24 ± 1.72	7	-0.08	.110	1
C. sabulosa	+	0		1	3.32			1	4.58				
	—	0		1	-.98			0					
	all	5	.97 ± .44	6	.62 ± .69	6	2.18 ± .39	6	1.92 ± .37	22	0.22	.424*	18
P. californica	+	3	1.64 ± .44	4	1.43 ± .71	4	2.14 ± .40	5	1.85 ± .45				
	—	0		2	-1.00 ± .33	0		0					
	all	5	5.72 ± 1.75	5	5.29 ± .62	5	3.29 ± 1.15	7	1.52 ± 1.40	22	-0.77	-.591**	35
P. badia	+	3	5.94 ± .72	5	5.29 ± .62	5	3.29 ± 1.15	4	3.06 ± 1.14				
	—	0		0		0		1	-1.40				
	all	3	.93 ± .99	3	2.60 ± .83	1		1		8	0.04	.034	00
N. cinctipes	+	1	2.55	0		0		1	2.83				
	—	1	-.88	0		1	3.13	0					
	all	4	16.78 ± 8.34	4	4.35 ± .30	4	4.48 ± 3.97	8	-2.14 ± 5.54	20	-2.47	-.622**	39
A. signata	+	2	29.20 ±10.18	0		1	2.25	0					
	—	0		0		0		0					
	all	4	1.26 ± .54	3	.67 ± .25	5	-.38 ± .51	5	.18 ± .43	17	-0.20	-.485*	24
A. pacifica	+	1	2.38	0		1	1.38	0					
	—	1	-.20	0		3	-.94 ± .44	1	-1.14				
	all	2	1.47 ± .02	2	1.06 ± .75			2	2.86 ± 3.38	6	-2.17	-.307	9
C. sabulosa	+	1	1.49	1	1.37			1	6.23				
	—	0		0				0					

†$\bar{m}$ = average percentage in weight/week (m x 100)
††m = change in rate/degree C
*significantly correlated, $p > .05$
**significantly correlated, $p > .01$

signata in the laboratory was significantly correlated inversely with temperature, but other factors were apparently more important in determining the life span of the other species (Table 4).

The average growth rate of stoneflies kept in the laboratory was considerably less than that of wild ones in the stream, for the species that could be compared, although the fastest growing ones in the laboratory equaled the rate of those in nature. The initial growth rate, for the first two months of life in the laboratory, was greater than later on and was significantly correlated inversely with temperature for *Pa. badia* and *Ar. signata*, about 30 to 40 percent of the effect ($r^2 \times 100$) being attributable to temperature (Table 9). Longer-term growth was less influenced by temperature for these species (Table 9), suggesting that those individuals most affected by temperature did not survive much longer than about two months. Growth of *Ac. pacifica* was similarly affected inversely by temperature, but to a lesser extent (only 24 percent of the effect on initial growth rate being attributable to temperature, Table 9) and apparently not enough to cause early death.

The growth rate of *Ps. californica*, particularly initially, was increased at higher temperatures (Table 11). It was also the longest-lived species in the laboratory (Table 4).

The wide range of responses to the stress imposed by laboratory conditions (including temperature) is remarkable but not unexpected. The Plecoptera observed here came from mountain streams that naturally change considerably from season to season and along their course. Variation in ability to respond to stressful, changing environmental conditions would be of advantage to species living under such conditions: it would reduce the probability that all individuals would be eliminated. Such wide variation means that laboratory experiments such as this should use a large sample size of comparable individuals. It probably is not valid to compare animals from different locations or ones collected at different seasons, and Ludwig (1928) has demonstrated that different stages of development of an insect vary in sensitivity to temperature.

This study suggests that some species (i.e., *Ps. californica*, *Pa. badia*, *Ac. pacifica*, and perhaps *C. sabulosa*) were better suited to long-term observations than were the others. Variables other than temperature had pronounced effects on stonefly growth and fate, and these should be elucidated before further studies are undertaken. The most important of these seem, subjectively, to be water quality, the nature of water movement in the habitat relative to energy expenditure by the animal, suitability of the habitat to the animal's behavior, and (most important) food. The physiological state of individuals is more difficult to assess but must be considered.

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